19. The method of claim 8, wherein in step (ii), prior to centrifugation, at least one protease inhibitor is added.

B3 cont'd.

20. The method of claim 8, wherein the anionic detergent is 10-30% sarkosyl.

21. The method of claim 8, wherein in step (ii), the centrifugation is carried out after depositing the suspension containing the PrPres on a 6-20% sucrose cushion.--

REMARKS

The Office Action dated October 10, 2002 has been carefully reviewed and the forgoing amendments are made in response thereto. In view of these amendments and the following remarks, Applicants respectfully request reconsideration and reexamination of this application and the timely allowance of the pending claims.

Applicant respectfully submits that no new prohibited matter has been introduced by the amendments. Written description support for the amended claims can be found throughout the specification, in the original claims and in the claims as amended on July 13, 1999. Additionally, support for the amendments to claims 8, 12 and 13, as well as support for new claims 16-21, can be found in the specification as indicated in the chart below.

Claims(s)	Support in specification
8	page 8, lines 3-35
12, 13	page 9, lines 8-22
16, 17	page 9, lines 3-6
18	page 8, lines 13-15
19, 21	page 9, lines 15-22
20	page 8, lines 21-24

I. Summary of the Office Action

Claims 7-13, the claims of Group II, following a Restriction Requirement Office Action, are currently pending. Claims 1-6 (Group I) and 15 (Group III) are withdrawn on the grounds of being drawn to non-elected inventions.

Claims 7-13 have been rejected under 35 U.S.C. §103(a) as being unpatentable over McKenzie *et al.*, *J Virol* 68(11):7534-7536, 1994, and Prusiner *et al.*, U.S. Patent No. 5,834,593.

II. Response to the Office Action

Rejections under 35 U.S.C. §103(a)

1. Claims 7-13 have been rejected under 35 U.S.C. §103(a), purportedly for being unpatentable over McKenzie *et al.*, *J Virol* 68(11):7534-7536, 1994, hereinafter referred to as McKenzie. The Examiner notes that McKenzie provides methods for the isolation and purification of PrPres, but does not disclose every single step of Applicants' methods. Respectfully, the feature of a single step differentiates Applicants' claimed purification method from that of McKenzie. On page 7535 of this reference, lines 2-8 of the right-hand column, McKenzie states that to purify PrPres for analysis from brain homogenates, the method of Bolton *et al.* ("Isolation and structural studies of the intact scrapie agent protein," *Arch Biochem Biophys* 258(2):579-590, 1987, hereinafter referred to as Bolton), a copy of which is enclosed, was used with one modification. The proteinase K digestion step, which in McKenzie and in Bolton follows several centrifugation steps, was modified. On pages 580 and 581 of Bolton, the first three paragraphs under "Materials and Methods," and on page 583, the paragraph under "Fig. 3," the purification procedure is described, which entails at least five centrifugation steps and treatment with RNase and DNase between the third and fourth centrifugation steps. In the first step, preparation of brain homogenates, tissue is homogenized in a buffer containing Tris-HCl, EDTA, NaCl, DTT and sarkosyl, but no protease is added. The homogenate is spun, the pellet discarded, and the supernatant is further purified in centrifugation/pellet resuspension steps. No protease is added in these preparative steps.

In contrast, claim 8 as amended recites a purification method consisting essentially of only one centrifugation step that is required for purification. In Applicants' method, after the tissue sample is homogenized, NaCl is added, as well as a solution comprising a protease and an anionic detergent. The combination of the salt, the protease and the detergent promote the aggregation of the PrPres so that it is spun out of solution at relatively low speed, *e.g.*, 25,000 – 30,000 g for 1-2 hours, at 16-22°C. This combination of reagents and centrifugation conditions

→ NOT IN CLAIMS

is critical and allows for the purification of PrPres in a single-centrifugation method that is both reliable and sensitive.

In the method of McKenzie, after centrifugation of the homogenate (without protease) at 22,500 g for half an hour, at 4°C, the PrPres remains in solution and is pelleted only by spinning at 150,000 g for 3 hours, at 4°C. Additionally, the multiple centrifugation steps required in the method of McKenzie significantly reduce the yield of PrPres obtained in the final sample. This loss defeats an important purpose of the procedure, which is to process biological samples for PrPres detection.

Similarly to Bolton, Xi et al. ("Detection of proteinase-resistant protein (PrP) in small brain tissue samples from Creutzfeldt-Jakob disease patients," *J Neurol Sci* 24:171-173, 1994), a copy of which is enclosed, describe a method in which PrPres is purified from brain tissue by first spinning a brain homogenate containing detergent at low speed (22,000 g for 20 min., at 10°C) and then centrifuging the supernatant obtained at 215,000 g for 2 hours at 10°C to pellet the PrPres. Two additional centrifugation steps are carried out, followed by treatment with protease (see the abstract on page 171 and page 172, left-hand column).

Thus, McKenzie teaches away from Applicants' method, and one looking for a simple and rapid method of purifying PrPres from animal tissue would not look to the method of McKenzie. McKenzie provides no guidance for developing a simpler or more rapid procedure for PrPres purification than the method of Bolton. The teachings of McKenzie do not disclose or suggest all the limitations of claim 8 as amended and would provide no guidance to one skilled in the art as to how to purify PrPres from a tissue sample in a simple method with one centrifugation step. Applicants also disagree with the Examiner's allegation in part 5 of the Office Action that "all those steps" not taught in McKenzie "are well known in the art and would only require routine experimentation." This statement is not supported, and the sort of experimentation required is not explained- experimentation to make the method work, experimentation for optimization, etc. The Examiner has not presented grounds adequate to establish a *prima facie* case of obviousness in view of the cited reference.

Accordingly, claim 8 and its dependent claims 12, 13 and 16-21, as amended, cannot be considered unpatentable in view of McKenzie, and Applicants respectfully request that this rejection be withdrawn.

2. Claims 7-13 have also been rejected under 35 U.S.C. §103(a) purportedly for being unpatentable over Prusiner *et al.*, U.S. Patent No. 5,834,593, hereinafter referred to as Prusiner. Respectfully, Prusiner is concerned with genetic engineering methods to produce soluble forms of PrP^{Sc} and does not disclose methods of isolating or purifying PrPres from biological samples. Rather, column 1, line 46, through column 2, line 7, of Prusiner notes that the natural form of PrP^{Sc} is difficult to work with because it is insoluble, and for uses of the protein, such as generating antibodies and developing drug screening assays, soluble forms of PrP^{Sc} should be prepared. Consequently, one seeking to work with natural forms of PrP would turn to sources other than Prusiner. In column 4, lines 1-31, a test involving centrifugation of a genetically modified PrP^{Sc} at 100,000 g for one hour, to determine whether or not a modified protein can be considered "soluble" is described, but this test is not part of or related to a method for purifying PrPres from a biological sample. Moreover, in the centrifugation test of Prusiner, the PrP^{Sc} is placed in water, a salt solution or a mild detergent, but no protease is added.

Thus, the teachings of Prusiner do not disclose or suggest all the limitations of claim 8 as amended, nor do they provide guidance to one skilled in the art for developing a method for purifying PrPres from a tissue sample using essentially one centrifugation step. Accordingly, the instant claims, as amended, cannot be considered unpatentable in view of Prusiner, and Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

The foregoing amendments and remarks are being made to place the application in condition for allowance. Applicants respectfully request reconsideration and the timely allowance of the pending claims. A favorable action is awaited. Should the Examiner find that an interview would be helpful to further prosecution of this application, he is invited to telephone the undersigned at his convenience.

Except for fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-0310. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 C.F.R. § 1.136(a)(3).

Respectfully submitted,
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Dated: April 10, 2003

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Version with markings to show changes made

In the claims:

8. (twice amended) A method of isolating PrPres, from an organ or a tissue, in particular the spleen or the brain, [which is capable of being used in a method according to Claim 1, characterized in that it comprises essentially the following steps] consisting essentially of:

(i) homogenization of organ or tissue, collected after sacrificing the animal, by mechanical grinding in a homogenization buffer, followed by the addition, to the homogenate obtained, of a salt having a high ionic strength and capable of promoting the aggregation of the PrPres in a 1:1 (v/v) ratio, followed by calibration of the homogenate, for the production of a homogenate comprising, in weight/volume, from 5 to 50% of the said organ or tissue;
(ii) specific extraction of PrPres by treating the homogenate obtained in step (i) by incubating the suspension obtained with a solution comprising a protease and an anionic detergent capable of promoting the aggregation of the PrPres, and a single separation of the PrPres, by centrifugation at 25,000-60,000 g.h., for example at 25,000-30,000 g for 1 to 2 h, preferably at 16-22°C, of the suspension obtained, deposited on a buffer cushion having a density of between 1.02 and 1.08, at 20°C and recovering the centrifugation pellet comprising the said PrPres; and, if necessary,

(iii) purification of the PrPres by suspending the centrifugation pellet obtained in (ii) in a Laemmli buffer comprising 1-5% SDS, incubating in this buffer at 100°C for 2-10 minutes and centrifuging at 12,000-15,000 g for 10-15 minutes at 16-22°C.

12. (twice amended) The [A] method according to Claim 8, wherein [characterized in that] during the extraction step (ii) the solution used for the extraction comprises an anionic detergent capable of promoting the aggregation of the PrPres and a zwitterionic detergent, such as a sulphobetaine, preferably the sulphobetaine SB 3-14 at 1-2%, in a 1:1 (v/v) ratio.

13. (twice amended) The [A] method according to Claim 8, wherein [characterized in that] in the extraction step (ii) the centrifugation is [preferably] carried out after depositing the suspension containing the PrPres on a cushion comprising, in a mixture, 6-20% sucrose and a sulphobetaine.